

# Workshop on contrast enhancement

Saturday 14th February 2026

## Dark-ground illumination

Transmitted dark-ground illumination works by blocking the light that would normally pass through the specimen into the objective, so that the specimen is illuminated only by light from the periphery of the condenser. This results in a specimen that glows brightly against a black background.

Dark-ground illumination can be produced with circular black stops placed in the filter tray of a normal substage condenser. The circular black stops can be made at home, or purchased on eBay.

Microscope manufacturers sell dry dark-ground condensers for use with objectives with an NA up to about 0.65.

For higher NA objectives, an immersion dark-ground condenser is needed, with oil bridging the gap between the top lens of the condenser and the bottom of the slide. Immersion objectives also need to be oiled to the top of the slide.

Dark-ground illumination can provide a dramatic increase in contrast for transparent specimens such as beetle or fly wings or unstained specimens. Bright lamps are needed because most of the light is blocked by the central stop. Diaphragms in the light path need to be wide open, so flare can be a problem, and slides need to be very clean because every dust speck is brightly illuminated.

Dark backgrounds can also be produced with crossed polarisers, or with black material below specimens and incident illumination.

I have brought an Olympus dark-ground condenser for a compound microscope, an Olympus Abbe condenser with a set of dark-ground stops (from eBay), and an Olympus dark-ground stop for their low-end condensers with 10×, 20× and perhaps 40× objectives.

I have also brought a large dark-ground condenser for an Olympus stereo microscope that needs a lot of light and has a small field of view (9 mm), and a simple set-up with an inverted ring-light and a pudding basin that has a wider field of view and has plenty of light.

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## Phase contrast

If you browse the catalogues of the major microscope manufacturers, you will see that there is more than one type of phase contrast. It can be either positive (the more common form, with the specimen darker than the background) or negative (with the specimen lighter than the background), but in both cases differences in refractive index within the specimen cause phase shifts which are made visible by changing them to differences in intensity.

Phase contrast can also be tailored to the contrast of the specimen, to suit specimens of low, normal or high contrast.

There is more than one way to achieve these variations, but in the Olympus equipment that I am familiar with, there is only one annulus in the condenser for objectives of the same magnification. The variations are found in the phase plate in the objectives. In positive phase contrast, the phase of diffracted light is retarded by  $90^\circ$ . In negative phase contrast, the phase of undiffracted surround light is retarded by  $90^\circ$ . To tailor the objectives to specimens of different contrasts, neutral density is added to either the diffracted light or the surround light.

Olympus used to manufacture four types of phase contrast objectives for the E, F, BH and BH-2 microscopes:

- **PLL** (Positive Low Low), for specimens of high contrast, subject appears darker than background
- **PL** (Positive Low), for specimens of relatively high contrast, subject appears darker than background
- **NM** (Negative Medium), for specimens of average contrast, subject appears lighter than background,
- **NH** (Negative High), for specimens of relatively low contrast, subject appears lighter than background

The two photographs of a cross-section of the stem of sunflower with the PLL and NH objectives illustrate the two extremes of phase contrast.

There are also four photographs of a transverse section of ileum (final part of small intestine), taken with PLL, PL, NM and NH objectives.

I have brought an Olympus phase contrast condenser, one objective, a phase telescope (used to help align the rings in the condenser and objective) and a green filter.

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## **Crossed polarisers and film retarders**

Colours can be made to appear in all sorts of materials and organisms when transmitted light is polarised.

As a minimum, you need two polarising filters, one below the specimen (called the polariser) and one above the specimen (called the analyser).

For the polariser, you can use a linear polarising filter for a camera, placed on top of the light output. It must be possible to rotate the polariser.

For the analyser, if the head of your microscope can be removed, a disc of polarising film can usually be inserted below the head.

You can also buy expensive polarisers and analysers from microscope manufacturers, but will probably not see much difference.

Manufacturers also sell expensive retarders (wave plates) that change the colours.

However, many (but not all) plastic films can be used as retarders by placing them on top of the polariser. Rotate the retarder and the colours will change. Sources of plastic film include wrapping of greetings cards and CDs, and windows in cake boxes. You can mount the retarders in frames for 35mm slides, or make holders from cereal boxes or other thin card.

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